EXHIBIT C

1: Stem Cell Rev. 2007;3(1):30-8.

Links

Networking of WNT, FGF, Notch, BMP, and Hedgehog Signaling Pathways during Carcinogenesis.

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The biological functions of some orthologs within the human genome and model-animal genomes are evolutionarily conserved, but those of others are divergent due to protein evolution and promoter evolution. Because WNT signaling molecules play key roles during embryogenesis, tissue regeneration and carcinogenesis, the author's group has carried out a human WNT-ome project for the comprehensive characterization of human genes encoding WNT signaling molecules. From 1996 to 2002, we cloned and characterized WNT2B/WNT13, WNT3, WNT3A, WNT5B, WNT6, WNT7B, WNT8A, WNT8B, WNT9A/WNT14, WNT9B/WNT14B, WNT10A, WNT10B, WNT11, FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD10, FRAT1, FRAT2, NKD1, NKD2, VANGL1, RHOU/ARHU, RHOV/ARHV, GIPC2, GIPC3, FBXW11/betaTRCP2, SOX17, TCF7L1/TCF3, and established a cDNA-PCR system for snap-shot and dynamic analyses on the WNT-transcriptome. In 2003, we identified and characterized PRICKLE1, PRICKLE2, DACT1/DAPPER1, DACT2/DAPPER2, DAAM2, and BCL9L. After completion of the human WNT-ome project, we have been working on the stem cell signaling network. WNT signals are transduced to beta-catenin, NLK, NFAT, PKC, JNK and RhoA signaling cascades. FGF20, JAG1 and DKK1 are target genes of the WNT-beta-catenin signaling cascade. Cross-talk of WNT and FGF signaling pathways potentiates beta-catenin and NFAT signaling cascades. BMP signals induce IHH upregulation in co-operation with RUNX. Hedgehog signals induce upregulation of SFRP1, JAG2 and FOXL1, and then FOXL1 induces BMP4 upregulation. The balance between WNT-FGF-Notch and BMP-Hedgehog signaling networks is important for the maintenance of homoestasis among stem and progenitor cells. Disruption of the stem cell signaling network results in pathological conditions, such as congenital diseases and cancer.

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1: Lab Invest. 2005 Jun;85(6):747-55.

Vascular leakage in chick embryos after expression of a secreted binding protein for fibroblast growth factors.

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Fibroblast growth factors (FGFs) have been implicated in a variety of physiologic and pathologic processes from embryonic development to tumor growth and angiogenesis. FGFs are immobilized in the extracellular matrix of different tissues and require release from this storage site to trigger a response. Secreted FGF-binding proteins (FGF-BPs) can release immobilized FGFs, enhance the activity of locally stored FGFs and can thus serve as an angiogenic switch molecule in cancer. Here, we report on the effect of human FGF-BP transgene expression in chicken embryos. To establish the transgenic model, plasmid-based reporter vectors expressing luciferase, beta-galactosidase or green fluorescent protein were introduced through different routes into 4- to 5-day-old embryos grown outside their egg shell on top of the yolk sac. This allows for easy manipulation and continuous observation of phenotypic effects. Expression of human FGF-BP induced dose-dependent vascular permeability, hemorrhage and embryonic lethality. Light and electron microscopic studies indicate that this hemorrhage results from compromised microvascular structure. An FGF-1 expression vector with an added secretory signal mimicked this vascular leakiness phenotype whereas wild-type FGF-1 required coexpression of a threshold amount of FGF-BP. This model is a powerful tool for real-time monitoring of the effects of transient transgene expression during embryogenesis.

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1: Curr Top Dev Biol. 2005;69:101-38.

Links

FULL-TEXT ARTICLE

Classical embryological studies and modern genetic analysis of midbrain and cerebellum development.

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The brain is a remarkably complex anatomical structure that contains a diverse array of subdivisions, cell types, and synaptic connections. It is equally extraordinary in its physiological properties, as it constantly evaluates and integrates external stimuli as well as controls a complicated internal environment. The brain can be divided into three primary broad regions: the forebrain, midbrain (Mb), and hindbrain (Hb), each of which contain further subdivisions. The regions considered in this chapter are the Mb and most-anterior Hb (Mb/aHb), which are derived from the mesencephalon (mes) and rhombomere 1 (r1), respectively. The dorsal Mb consists of the laminated superior colliculus and the globular inferior colliculus (Fig. 1A and B), which modulate visual and auditory stimuli, respectively. The dorsal component of the aHb is the highly foliated cerebellum (Cb), which is primarily attributed to controlling motor skills (Fig. 1A and B). In contrast, the ventral Mb/aHb (Fig. 1B) consists of distinct clusters of neurons that together comprise a network of nuclei and projections-notably, the Mb dopaminergic and Hb serotonergic and Mb/aHb cholinergic neurons (Fig. 1G and H), which modulate a collection of behaviors, including movement, arousal, feeding, wakefulness, and emotion. Historically, the dorsal Mb and Cb have been studied using the chick as a model system because of the ease of performing both cell labeling and tissue transplants in the embryo in ovo; currently DNA electroporation techniques are also used. More recently the mouse has emerged as a powerful genetic system with numerous advantages to study events underpinning Mb/aHb development. There is a diverse array of spontaneous mutants with both Mb- and Cb-related phenotypes. In addition, numerous gene functions have been enumerated in mouse, gene expression is similar across vertebrates, and powerful genetic tools have been developed. Finally, additional insight into Mb/aHb function has been gained from studies of genetic diseases, such as Parkinson's disease, schizophrenia, cancer, and Dandy Walker syndrome, that afflict the Mb/aHb in humans and have genetic counterparts in mouse. Accordingly, this chapter discusses a spectrum of experiments, including classic embryology, in vitro assays, sophisticated genetic methods, and human diseases. We begin with an overview of Mb and aHb anatomy and physiology and mes/r1 gene expression patterns. We then provide a summary of fate-mapping studies that collectively demonstrate the complex cell behaviors that occur while the Mb and aHb primordia are established during embryogenesis and discuss the integration of both anterior-posterior (A-P) and dorsal-ventral (D-V) patterning. Finally, we describe some aspects of postnatal development and some of the insights gained from human diseases.

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1: Mech Dev. 2001 Mar;101(1-2):293-7. Links

Embryonic expression patterns of the mouse and chick Gas1 genes.

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Control of cell proliferation is essential to generate the defined form of a multi-cellular organism. While much is known about the regulators for cell cycle progression, relatively little is known about the state of growth arrest. Growth arrest (G0) is defined as a cell in a metabolically active but proliferation-quiescent state (reviewed in Baserga (1985) The Biology of Cell Reproduction), typically induced by serum starvation in vitro. Using subtractive hybridization, Schneider et al. (Cell 54 (1988) 787) identified six genes (Gas1 through Gas6) whose expressions are upregulated in serum-deprived NIH3T3 cells. Among the Gas genes, Gas1 is the only one that can cause growth arrest when expressed in cultured cell (Cell 70 (1995) 595; Int. J. Cancer 9 (1998) 569). Here, we describe for the first time the expression pattern of Gas1 during mouse embryogenesis. Our data reveal that Gas1 is expressed in many regions that the cells are actively proliferating and suggest that it may have other roles during development than negatively regulating cell proliferation. Furthermore, we have cloned the chick GAS1 gene and documented the similarity and divergence of Gas1 gene expression patterns between the two species.

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1: Dev Dyn. 2006 Mar;235(3):681-90.

Differential inhibition of Wnt-3a by Sfrp-1, Sfrp-2, and Sfrp-3.

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Secreted frizzled related proteins (Sfrps) are extracellular attenuators of Wnt signaling that play important roles in both embryogenesis and oncogenesis. Although Sfrps are generally thought to bind and sequester Wnts away from active receptor complexes, very little is known about the specificity of Sfrp family members for various Wnts. In the developing chick neural tube, sfrp-1, 2, and 3 transcripts are expressed in and adjacent to the dorsal neural tube, where Wnt-1 and Wnt-3a are expressed. To better define the possible roles of Sfrp-1, 2, and 3 in the neural tube, we first tested the ability of purified Sfrps to inhibit Wnt-3a-induced accumulation of beta-catenin in L cells. We find that both Sfrp-1 and Sfrp-2 can inhibit Wnt-3a activity while Sfrp-3 cannot. To determine where Sfrp-1 and Sfrp-2 impinge on the Wnt signaling pathway, we tested the ability of these Sfrps to inhibit Wnt signaling induced by the addition of LiCl, an inhibitor of GSK-3. Sfrp-1 and Sfrp-2 are unable to inhibit the accumulation of beta-catenin in LiCl-treated cells, suggesting that the ability of Sfrps to inhibit the accumulation of beta-catenin is GSK-3 dependent. We have further shown that Sfrp-2 inhibits the ability of ectopic Wnt-3a to stimulate proliferation in the developing chick neural tube. These results provide the framework for understanding how Sfrps function to regulate Wnt-3a activity in developing embryos and in cancer.

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1: Differentiation. 1995 Jun;58(5):313-20.

Expression of gicerin in development, oncogenesis and regeneration of the chick kidney.

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Neurite outgrowth factor, which promotes neurite extension from neuronal cells, is an extracellular matrix glycoprotein belonging to the laminin family. Gicerin is a protein that binds neurite outgrowth factor. Its cDNA cloning has revealed that it is a novel cell adhesion molecule belonging to the immunoglobulin super-family. Functional analysis demonstrates that gicerin possesses homophilic binding activity as well as heterophilic binding activity with neurite outgrowth factor. We examined the role and expression of neurite outgrowth factor and gicerin in chick kidney during development. In the embryonic kidney, gicerin was found to be highly expressed both on ureteric bud cells and metanephrogenic mesenchymal cells, when the mesenchymal cells become condensed to be converted into polarized epithelial cells. In the adult kidney, the expression of gicerin was decreased and restricted to the glomerulus, proximal tubule and medullary loop. On the other hand, neurite outgrowth factor was constitutively expressed in the basement membranes of tubules and the matrices of glomeruli during development. As some molecules which are expressed during embryogenesis and suppressed after maturation are re-expressed in tumor cells or tissues during regeneration, we also examined the expression of gicerin in chicken Wilms' tumor and regenerating kidney in interstitial nephritis. Gicerin was remarkably upregulated in Wilms' tumor and re-expressed in collecting ducts recovering from interstitial nephritis. These findings suggest that gicerin could play a role not only in normal renal development but also in oncogenesis and regeneration.

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